ADCHEM 2006

International Symposium on Advanced Control of Chemical Processes
Gramado, Brazil – April 2-5, 2006

ROBUST ADAPTIVE CONTROL OF YEAST FED-BATCH CULTURES

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Abstract: A robust controller combining a feedforward compensator (for the measured disturbance) and a feedback *RST* controller is designed for the control of *S. cerevisiae* cultures. The controller is based on the linearization of Sonnleitner's model allowing a simple transfer function model to be derived, which describes the relation between the ethanol concentration, the substrate feed and a measurable disturbance - image of the substrate demand for cell growth. This control scheme is made robust to neglected high frequency dynamics (of glucose and oxygen) and uncertain stoichiometry coefficients using the observer polynomial. This control scheme, whose performance is demonstrated in simulation, requires only the on-line measurements of the ethanol concentration and bioreactor volume, estimation of the oxygen transfer rate, and minimal *a priori* process knowledge, i.e. only one stoichiometric coefficient. *Copyright 2006 IFAC.*

Keywords: Robust control; Adaptive control; Fed-batch fermentation; Biotechnology;

1. INTRODUCTION

Due to their robustness and ability to utilize cheap materials for growth and production, *Saccharomyces cerevisiae* strains are among the most popular industrial microorganisms. Recently, with the achievement of modern gene technology, *S. cerevisiae* have been increasingly used as host organisms for producing recombinant proteins (production of insulin, vaccines, ...).

To ensure optimal operating conditions (i.e. to maximize biomass productivity), a commonly used method consists in regulating the ethanol concentration at a low value. Several control schemes, which have been tested in genuine industrial applications, have been proposed to this end (see, e.g. Chen *et al.*, 1995; Pomerleau, 1990; Pomerleau and Viel, 1992; Axelsson, 1988). These controllers result from the linearizing control theory (Bastin and Dochain, 1990), where the control law can be viewed as a proportional controller acting around a trajectory representing the substrate demand for cell growth. In (Chen *et al.*, 1995), this trajectory is deduced from an on-line biomass estimation while in (Pomerleau, 1990), it is deduced from the measurement of the oxygen transfer rate (*OTR*). In this connection, nonlinear parameter adaptation techniques are used to estimate uncertain parameters.

In this contribution, a simplified model is derived by linearization of the global nonlinear model of Sonnleitner and Käppeli (1986) around the above-mentioned trajectory. This transfer-function model depicts the relationship between the ethanol concentration and the substrate feed and a measured disturbance representing the substrate demand for cells growth. An adaptive feedforward controller is used in combination with a feedback RST controller to regulate the ethanol concentration. This approach allows the use of linear control theory and a simple design of a robust controller. For instance, requirements in terms

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of measurement noise attenuation or robustification to neglected high frequency dynamics can easily be incorporated. In comparison with the linearizing control theory, the purely linear control framework, into which the suggested control algorithm is developed, allows the robustness issues to be easily taken into account.

2. MODELING OF YEAST FED-BATCH CULTURES

2.1 *Nonlinear dynamic model*

The metabolism of yeast depends strongly on the culture conditions. During the aerobic growth, glucose and ethanol can be used as carbon sources according to the following reaction scheme :

$$\text{Glucose oxidation :} \quad \mathrm{G} + k_5\,\mathrm{O} \xrightarrow{r_1} k_1\,\mathrm{X} + k_7\,\mathrm{P} \quad (1a)$$

$$\text{Glucose fermentation :} \quad \mathrm{G} \xrightarrow{r_2} k_2\,\mathrm{X} + k_4\,\mathrm{E} + k_8\,\mathrm{P} \quad (1b)$$

$$\text{Ethanol oxidation :} \quad \mathrm{E} + k_6\,\mathrm{O} \xrightarrow{r_3} k_3\,\mathrm{X} + k_9\,\mathrm{P} \quad (1c)$$

where X, G, E, O, P are, respectively, the concentration in the culture medium of biomass, glucose, ethanol, dissolved oxygen and dissolved carbon dioxide, and k_i are the pseudo-stoichiometric coefficients.

The reaction rates associated with these reactions are :

$$r_1 = \min\left(r_G, \frac{r_{Omax}}{k_5}\right) \quad (2)$$

$$r_2 = \max\left(0, r_G - \frac{r_{Omax}}{k_5}\right) \quad (3)$$

$$r_3 = \max\left(0, \min\left(r_E, \frac{r_{Omax} - k_5 r_G}{k_6}\right)\right) \quad (4)$$

The kinetic terms associated with the glucose consumption r_G, the respiratory capacity r_{Omax} and the potential ethanol oxidative rate r_E are :

$$r_G = \mu_G \frac{\mathrm{G}}{\mathrm{G} + K_G},\ r_{Omax} = \mu_O \frac{\mathrm{O}}{\mathrm{O} + K_O},\ r_E = \mu_E \frac{\mathrm{E}}{\mathrm{E} + K_E}$$

where μ_G, μ_O and μ_E are the maximal values of specific growth rates, K_G, K_O and K_E are the saturation constants of the corresponding substrate.

This kinetic model is based on the bottleneck hypothesis developed by Sonnleitner and Käppeli (1986). It assumes a limited oxidation capacity of yeast, leading to the formation of ethanol under conditions of oxygen limitation and/or high glucose concentration. The glucose concentration corresponding to the oxidative capacity is denoted G_{crit}, and is such that $r_G = r_{Omax}/k_5$. According to the glucose concentration value, two different operating regimes can be distinguished. At low glucose concentrations ($G \leq G_{crit}$), the system is in respiratory regime. The glucose consumption rate is smaller than the maximal respiratory capacity ($r_G \leq r_{Omax}/k_5$) and the rate of the oxidative glucose metabolism is determined by the glucose consumption rate (2). Ethanol can be oxidized in parallel with glucose and the rate of the oxidative ethanol metabolism depends on the excess of respiratory capacity and the available ethanol (4). At high glucose concentrations ($G \geq G_{crit}$), the system is said in respiro-fermentative regime. The glucose consumption rate is larger than the maximal respiratory capacity ($r_G \geq r_{Omax}/k_5$) and the respiratory capacity of the cells determines the rate of the oxidative glucose metabolism (2). The excess of glucose is metabolized by the fermentative metabolism (3). Under oxygen starvation conditions, the fermentative metabolic pathway always predominates.

Based on the reaction scheme (1), the following macroscopic mass balances can be derived :

$$\frac{d(VX)}{dt} = (k_1 r_1 + k_2 r_2 + k_3 r_3) XV \quad (5a)$$

$$\frac{d(VG)}{dt} = -(r_1 + r_2) XV + F_{in} G_{in} \quad (5b)$$

$$\frac{d(VE)}{dt} = (k_4 r_2 - r_3) XV \quad (5c)$$

$$\frac{d(VO)}{dt} = -(k_5 r_1 + k_6 r_3) XV + V\,OTR \quad (5d)$$

$$\frac{dV}{dt} = F_{in} \quad (5e)$$

where G_{in} is the substrate concentration in the feed, F_{in} is the inlet feed rate, V is the culture medium volume and D is the dilution rate ($D = F_{in}/V$).

2.2 *Simplified linear model*

In (Valentinotti *et al.*, 2004), it is shown that maximization of biomass productivity corresponds to a feeding strategy which exactly fills the bottleneck. Hence, the optimal operating conditions correspond to the boundary between the respiro-fermentative and the respiratory regimes ($G = G_{crit}$). The nonlinear model (5) can be linearized around this optimal point, i.e. in the respiro-fermentative or respiratory regime, with $G \to G_{crit}$. For both regimes, it is assumed that there is no accumulation of glucose and oxygen in the culture medium so that the dynamics of the total amount of these substrates can be neglected. Along the optimal trajectory, where G is maintained at a low concentration ($G_{crit} \approx 0.02\,g/l$), glucose is the limiting substrate and the biomass assimilates very quickly the glucose fed to the bioreactor (so that $(r_1 + r_2)XV \approx F_{in} G_{in}$ and $d(VG)/dt \approx 0$). Moreover, the oxygen solubility in the culture medium is low and the dynamics associated to the oxygen transfer from the gaseous to the liquid phase is fast compared to the time constant of the biological process, so that the dynamics of the total amount of oxygen VO in the culture medium can be neglected ($d(VO)/dt \approx 0$).

In the respiro-fermentative regime, the nonlinear model is given by (5) where the rate of ethanol oxidation $r_3 = 0$. Based on (5b) and (5d), the previous assumptions yield :

$$\frac{d(VG)}{dt} \approx 0 \qquad \Rightarrow \qquad r_2 XV = F_{in} G_{in} - r_1 XV \quad (6)$$

$$\frac{d(VO)}{dt} \approx 0 \qquad \Rightarrow \qquad r_1 X = \frac{OTR}{k_5} \quad (7)$$

Substitution into (5c) gives (using also (5e)) :

$$\frac{dE}{dt} = \frac{F_{in}}{V}(k_4 G_{in} - E) - \frac{k_4}{k_5} OTR \quad (8)$$

The nominal trajectory is characterised by a constant ethanol concentration E^* and, in turn, by a dilution profile $D^* = F_{in}^*/V^*$ satisfying the following relation :

$$D^* = \frac{1}{k_4 G_{in} - E^*} \frac{k_4}{k_5} OTR \quad (9)$$

Linearizing (8) around the nominal trajectory and neglecting the variations of E and V gives :

$$\frac{dE}{dt} = F_{in} \frac{k_4 G_{in} - E^*}{V^*} - \frac{k_4}{k_5} OTR \quad (10)$$

For the respiratory operating regime, similar developments give :

$$\frac{dE}{dt} = F_{in} \frac{\frac{k_5}{k_6} G_{in} - E^*}{V^*} - \frac{OTR}{k_6} \quad (11)$$

Finally, for both operating regimes, the ethanol dynamics can be expressed by the same discrete transfer function :

$$E(k) = \frac{q^{-1}}{1 - q^{-1}} \left[b F_{in}(k) - \alpha OTR(k) \right] \quad (12)$$

where q^{-1} is the backward shift operator and the parameters b and α listed in Table 1 depend on the operating regime (T_s is the sampling time).

The block diagram of Fig. 1 shows the schematic representation of the simplified fed-batch fermentation model. The measured perturbation αOTR represents the glucose demand for cells growth. If the feeding flux $b F_{in}$ is higher or lower than the measured perturbation, there is production or consumption of ethanol, respectively.

Around the optimal trajectory, the system is modeled by the same transfer function for both operating regimes. The only difference lies in the b and α values which change with the operating regime. For controller design, the b variation according to the operating regime and the neglected dynamics of glucose and oxygen can be considered as modeling uncertainties, with respect to which the controller has to be robust. Moreover, the gain b evolving widely with the volume, a robust and adaptive control strategy is needed.

3. CONTROL STRATEGY

3.1 *Controller design*

Based on the linear model (12), where the notation $\hat{A}(q^{-1}) = 1 - q^{-1}$ and $\hat{B}(q^{-1}) = b\, q^{-1}$ is used, a linear controller can be designed. The control scheme

Table 1. Parameters expressions of linear discrete model (12).

	Respiro-fermentative regime	respiratory regime
b	$T_s \frac{k_4 G_{in} - E^*}{V^*}$	$T_s \frac{\frac{k_5}{k_6} G_{in} - E^*}{V^*}$
α	$T_s \frac{k_4}{k_5}$	$T_s \frac{1}{k_6}$

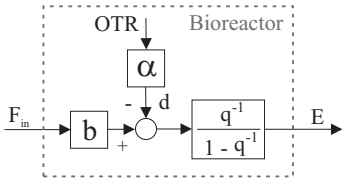

Fig. 1. Schematic representation of the simplified (linear) fed-batch fermentation model.

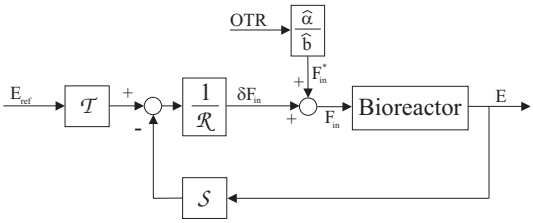

Fig. 2. Closed-loop diagram. A feedback RST controller is used in combination with an adaptive feedforward controller in order to cancel the measured disturbance d effect on the ethanol concentration E.

developed in this work consists of a feedback RST controller and a feedforward controller canceling the measured disturbance effect on the output. The corresponding block diagram is shown in Fig. 2. The feedback RST controller compensates deviations from the nominal trajectory defined by (12) when E is equal to a constant setpoint E_{ref} :

$$F_{in}^*(k) = \frac{\hat{\alpha}(k)}{\hat{b}(k)} OTR(k) \quad (13)$$

where $\hat{\alpha}(k)$ and $\hat{b}(k)$ are the adapted values of the corresponding parameters.

The control law is written as follows :

$$\mathcal{R}(q^{-1}) \delta F_{in}(k) = -\mathcal{S}(q^{-1}) E(k) + \mathcal{T}(q^{-1}) E_{ref}(k)$$

$$F_{in}(k) = \delta F_{in}(k) + \frac{\hat{\alpha}(k)}{\hat{b}(k)} OTR(k)$$

where δF_{in} is the controller output which represents the variation of the feed rate around the nominal trajectory F_{in}^*, E_{ref} is the desired ethanol setpoint and $\mathcal{R}$, $\mathcal{S}$ and $\mathcal{T}$ are polynomials in backward-shift operator q^{-1}.

For the sake of simplicity, the RST controller polynomials are computed by a pole-placement procedure (Astrom and Wittenmark, 1997). Let the reference model be given by :

$$H_m(q^{-1}) = \frac{B(q^{-1}) P(1)}{B(1) P(q^{-1})} \quad (14)$$

where $P(q^{-1})$ allows the poles of the tracking closed-loop transfer function to be chosen.

Let assume that the controller must ensure zero-steady-state error with respect to a step disturbance acting on the process input. This deterministic disturbance $d(k)$ can be modeled by the following dynamical system :

$$A_d(q^{-1})d(k) = C(q^{-1})\delta(k) \qquad (15)$$

where $\delta(k)$ is the unit pulse, $A_d(q^{-1}) = \Delta(q^{-1}) = 1 - q^{-1}$ and $C(q^{-1}) = T(q^{-1})$ is an observer polynomial, which can be used to robustify the control law as it is common practice for predictive controllers (see e.g. Soeterboek, 1992; Clarke, 1996).

With the previous reference and disturbance models, the following $\mathcal{R}$, $\mathcal{S}$ and $\mathcal{T}$ polynomials can be selected :

$$\mathcal{R} = \Delta P, \qquad \mathcal{S} = \mathcal{S}' P, \qquad \mathcal{T} = T\frac{P(1)}{\hat{B}(1)} \qquad (16)$$

where $\mathcal{S}'$ is the solution of $T = \Delta\hat{A} + \hat{B}\mathcal{S}'$.

Note that, in this way, an integrator is introduced in the controller. In practice, the integral action must be implemented with an anti-reset windup mechanism in case of saturation of the control action. In fact, at the beginning of a culture, the flow rate is very small and any deviation from the nominal trajectory can lead to negative flow rates which are not allowed. An anti-reset windup mechanism avoid amplifying oscillations when saturation occurs.

If the process model is correctly estimated, the closed loop transfer function can be written as follows :

$$\begin{aligned} E &= \frac{B\mathcal{T}}{A\mathcal{R}+B\mathcal{S}}E_{ref} + \frac{\mathcal{R}q^{-1}}{A\mathcal{R}+B\mathcal{S}}d \\ &= \frac{BP(1)}{\hat{B}(1)P}E_{ref} + \frac{\Delta q^{-1}}{T}d \end{aligned} \qquad (17)$$

Note that P does not appear in the transfer function related to the disturbance. Thus, the tracking and rejection behaviors can be adjusted independently in selecting the roots of the polynomials P and T, respectively. Note also that Δ appears in the numerator of the rejection transfer function. Therefore, the internal model principle (Francis and Wonham, 1976) ensures that a step of a process input disturbance will be rejected asymptotically by the controller (16).

The proposed controller can be tuned by selecting appropriate P and T polynomials. The overall time constant of the bioprocess is quite big and it is reasonable to choose a sampling time T_s of 0.1 h. The P polynomial is designed to achieve a first-order tracking behavior with a time constant of 1 h, i.e. P is given by $P(q^{-1}) = 1 - 0.9q^{-1}$. The observer polynomial T is designed in order to achieve a trade-off between the rejection performance and robustness. If the adapted gain $\hat{b}(k)$ corresponds to the actual process gain, the controller (16) yields a corrected open-loop transfer

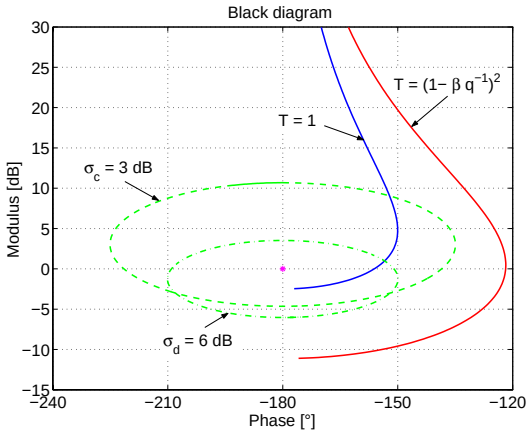

Fig. 3. Black diagram of the controlled system before robustification ($T(q^{-1}) = 1$) and after robustification ($T(q^{-1}) = (1-\beta q^{-1})^2$). σ_c and σ_d are the complementary and direct sensitivity functions.

function $\frac{B\mathcal{S}}{A\mathcal{R}}$, which depends only on the T polynomial, and whose stability robustness can be directly adjusted by choosing the T roots. The design rules of predictive controllers (see e.g. Soeterboek, 1992; Clarke, 1996) can be used and lead to $T(q^{-1}) = (1-\beta q^{-1})^2$. The robustness margin is monotonically increasing when the value of the parameter β increases. Obviously, the rejection performance decreases in the same way and, for this application, $\beta = 0.7$ leads to a good trade-off.

Fig. 3 shows the Black diagrams of control schemes without robustification ($T(q^{-1}) = 1$) and with robustification ($T(q^{-1}) = (1-\beta q^{-1})^2$). It is well known that ensuring a modulus lower than 6 dB for the direct sensitivity function σ_d and lower than 3 dB for the complementary sensitivity function σ_c provides a good stability robustness. It is apparent that, before robustification, the controller shows bad robustness at high frequencies, whereas a good robustness margin is required in this frequency range because the oxygen and glucose dynamics have been neglected. Robustification provides the desired margins.

In addition, robustification allows the influence of the noise corrupting the ethanol concentration measurement on the control signal to be strongly attenuated (noise on the control signal affects the pump wear).

3.2 *Adaptation scheme*

An indirect adaptation scheme is used. Considering (17), it can be shown that cancellation of the T polynomial in the tracking transfer function is effective only if the process gain is correctly estimated. Therefore, adaptation of the parameter b is required for constant tracking performance during a culture. The parameter b can be directly adapted using the measurements of E and V. The measurement of the volume can obtained from a measurement of the bioreactor weight. If a weight measurement is not available, the volume

can be estimated on-line from all liquid additions and withdrawals such as evaporation, sampling, base addition for pH control, etc. Therefore, the adapted b parameter is given by :

$$\hat{b}(k) = T_s \frac{k_4 G_{in} - E(k)}{V(k)} \tag{18}$$

Because, in practice, the α parameter is not known exactly, it must also be estimated on-line to ensure perfect cancellation of the measured disturbance. To this end, the discrete model (12) can be written as a linear regression :

$$\varepsilon_1(k) = \varepsilon_2(k)\,\alpha \tag{19}$$

where $\varepsilon_1(k) = \hat{b}(k-1)F_{in}(k-1) - A(q^{-1})E(k)$ and $\varepsilon_2(k) = OTR(k-1)$.

Linear regression (19) can be solved on-line by an appropriate algorithm (see e.g. Ljung, 1999) taking into account that ε_1 and ε_2 are corrupted with noise because these signals are computed from measurements. In practice, ε_1 and ε_2 are filtered by $P(1)/P(q^{-1})$ to remove the mutual correlation and a recursive least-squares (RLS) algorithm proves to give a satisfactorily unbiaised estimation of α.

Remark In (Pomerleau, 1990), the suggested nonlinear controller appears as a special case of the design procedure developed in this study. In fact, if $A_d(q^{-1}) = 1$, $C(q^{-1}) = 1$, and $T(q^{-1}) = 1$, i.e. there is no disturbance model nor observer polynomial, then the pole-placement procedure (Astrom and Wittenmark, 1997) based on the same reference model $H_m(q^{-1})$, gives :

$$\mathcal{R} = 1\,; \quad \mathcal{S} = \mathcal{T} = \frac{P(1)}{\hat{B}(1)} \tag{20}$$

Equations (14) can then be written as :

$$F_{in}(k) = \frac{\gamma\,(E_{ref}(k) - E(k)) + \hat{\alpha}(k)\,OTR(k)}{\hat{b}(k)} \tag{21}$$

where $\gamma = P(1)$.

This controller can be interpreted as a proportional controller acting around the nominal trajectory given by (13). In (Pomerleau, 1990), the parameter α is not estimated thanks to a RLS algorithm but with a nonlinear estimation technique. An observer based estimator (Bastin and Dochain, 1990) is designed based on the differential equation (8) and the observer parameters are tuned so as to make the estimation dynamics independent of the process dynamics (Perrier *et al.*, 2000). Note that the same nonlinear estimation technique could be used in the present study.

4. SIMULATION RESULTS

The control algorithm described in Section 3 is implemented in simulation on the nonlinear process model presented in Section 2.1. When the ethanol concentration is regulated to the setpoint E_{ref} = 1 g/l, the process operates in respiro-fermentative regime. Thus, the only non operational parameter required to compute the controller is the stoichiometric coefficient k_4. The value proposed by Sonnleitner and Käppeli (1986) is chosen, k_4 = 0.48 [g of E/g of G]. The operational parameters are given as follows : G_{in} = 500 g/l; V_0 = 5 l; X_0 = 0.4 g/l; E_0 = 0.5 g/l, where the subscript 0 denotes an initial value. The design polynomials for the controller are given by $P(q^{-1}) = 1 - 0.9\,q^{-1}$ and $T(q^{-1}) = (1 - 0.7\,q^{-1})^2$ so that the polynomials $\mathcal{R}$, $\mathcal{S}$ and $\mathcal{T}$ can be computed from (16).

A setpoint step is imposed at t = 10 h in order to evaluate the tracking performance. For simulation purposes, OTR is computed using a well established model of the oxygen transfer, i.e., $OTR = K_l a \cdot (O_{sat} - O)$, where O_{sat} is the saturation oxygen concentration and $K_l a$ is the volumetric mass transfer coefficient. A low value of $K_l a$ is chosen in order to simulate a substrate limitation by oxygen. In industrial yeast culture, this kind of limitation is very frequent because the oxygen transfer coefficient of industrial bioreactors is generally low. Thus, the controller must keep on performing well in this situation.

Simulation results are shown on Fig. 4 and 5, demonstrating that the control algorithm is able to regulate E at the setpoint. When the setpoint step occurs, the tracking behavior is in accordance with the reference model H_m (first order system with 1 h time constant). Fig. 5 shows that the maximum oxygen transfer rate is achieved at about 16 h. From that time on, the oxidation capacity decreases gradually as the biomass grows. In spite of this metabolism change, almost no deviation of the E signal from the setpoint is observed, i.e. the controller decreases the feed rate in order to prevent excessive formation of ethanol. As the OTR signal is a good image of the glucose demand for cell growth, the fluctuations of this demand can be instantaneously compensated to follow the nominal trajectory.

5. CONCLUSIONS

Linearization of Sonnleitner's model around a nominal trajectory allows a simple linear model of the fed-batch fermentation process of *Saccharomyces cerevisae* to be derived. This model describes the main macroscopic phenomena taking place in the bioreactor, i.e. it includes a transfer function between the substrate feed and the ethanol concentration, and a measured disturbance. This disturbance, which can be measured on-line via the OTR signal, is the image of the substrate demand for cell growth. As explained by the linear model, the production or consumption of ethanol results from the excess or deficit of feed rate applied to the system, as compared to the substrate demand for growth. The advantage of using a linear

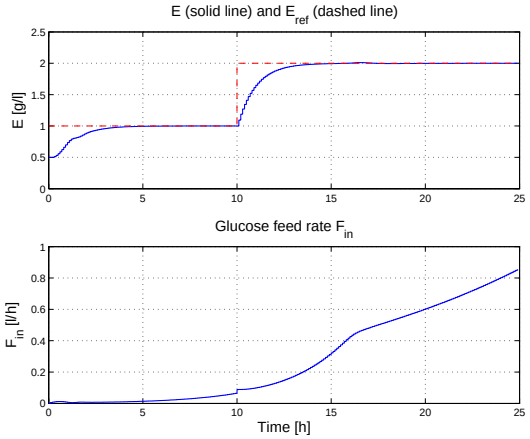

Fig. 4. Simulation results of a yeast fed-batch fermentation controlled with the algorithm proposed in Section 3. Ethanol concentration E and inlet flow rate F_{in}.

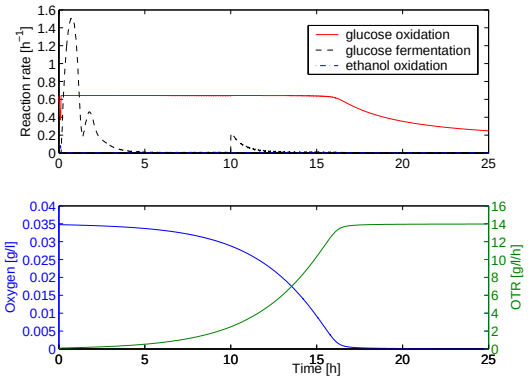

Fig. 5. Simulation results of a yeast fed-batch fermentation controlled with the algorithm proposed in Section 3. Reaction rates, dissolved oxygen concentration O and oxygen transfer rate OTR.

model (in contrast with the usual non linear models used for bioprocess simulation) is the possibility to easily develop a robustness analysis based on linear control theory.

In order to cancel the measured disturbance effect on the ethanol concentration, a feedforward controller is used in combination with a feedback RST controller. When developing the simplified process model, several high frequencies dynamics are neglected and the numerical values of some stoichiometric coefficients can be uncertain. Therefore, particular attention is paid to the design of a robust controller, allowing a separate (independent) tuning of the tracking performance and of the stability margins. As the process parameters can evolve during a culture, an indirect adaptive scheme is used to update on-line the controller parameters. The proposed control scheme can be viewed as a generalisation of the controller proposed in (Pomerleau, 1990).

The control algorithm requires only two on-line measurements : E and OTR. OTR measurements are commonly available and reliable E sensors are now becoming available at reasonable costs. Moreover, the V signal is also needed, which can be estimated by integration of all the liquid fluxes or via simple bioreactor weight measurements. With these on-line signals at hand, the implementation of control algorithm is quite simple. Tests in simulation are presented in the present paper, and these preliminary results demonstrate the ability of the controller to maintain the desired ethanol setpoint even when oxygen limitation occurs (as it is frequent in many industrial applications).

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